# Volatile Chemicals by Headspace GC/MS(EI)

## 1 Introduction

The analysis of volatile chemicals is performed by headspace gas chromatography. This technique is based on various gas laws which state that when a volatile liquid in solution comes into contact with a closed air space, an equilibrium forms between the liquid phase and the headspace. At a given temperature, the partial pressure of the volatile in the "headspace" is directly proportional to its concentration in solution. This method affords a means of analyte separation from the biological matrix and produces a ready-made vapor for chromatographic analysis. It should be noted that this is a modification of a procedure used routinely in the analysis of biological specimens for ethanol.

## 2 Scope

This procedure allows for the screening and confirmation of volatile chemicals that may be present in biological and non-biological samples.

## 3 Principle

An aliquot of sample or control is combined with internal standard and sodium chloride in a headspace vial. The vial is heated for 30 minutes and then the headspace is analyzed by gas chromatography with mass spectral detection (GC/MS).

## 4 Specimens

This procedure can be performed on a biological fluid such as: blood, serum, plasma, urine, vitreous humor, or tissue homogenate. When available, a minimum of 0.25 mL of specimen is used in this assay. This procedure may also be performed on foods, beverages, or unknown solid or liquid samples, although dilution may be required for samples with high amounts of volatile chemicals present.

## 5 Equipment/Materials/Reagents

Guidance for the preparation of reagents may be found in the *Preparation of Chemical Reagents* standard operating procedure (Tox 103).

a. Agilent Gas Chromatograph/Mass Spectrometer equipped with a headspace autosampler and a 30 m x 0.25 mm x 1.4 µm DB-624 column, or equivalent

b. 20-mL or 10-mL disposable headspace vials, magnetic caps, and crimper

c. Vortex mixer

d. Volumetric flasks (100-mL and 1000-mL)

e. Pipette (Adjustable or 0.5 mL fixed)

f. Sodium Chloride (NaCl) (ACS Reagent Grade)

g. Deionized Water

h. Routine laboratory supplies, including disposable pipettes, wooden sticks, test tube racks, graduated cylinders, etc.

i. Saturated sodium chloride solution (aka brine solution):
To a 500-mL volumetric flask, add 450 mL deionized water and 175 g sodium chloride. Gently heat with continuous stirring for at least one hour. Remove the stirbar, fill to volume with deionized water, and mix by inversion. A small amount of undissolved solid should remain in the bottom of the flask. Store in glass at room temperature. Stable for one year.

## 6 Standards and Controls

a. Methanol (Reagent Grade)

b. Ethanol (200 proof, pharmaceutical grade)

c. Isopropanol (HPLC Grade)

d. Acetone (HPLC Grade)

e. Acetonitrile (HPLC Grade)

f. Acetonitrile Internal Standard Solution (0.08% (w/v)):
Add 100 µL acetonitrile to about 90 mL deionized water in a 100-mL volumetric flask. Dilute to the mark with deionized water and mix thoroughly. Store at room temperature in a tightly sealed glass container. Stable for 6 months.

g. Volatile Injection Solution / Positive Control (0.01 % v/v of each component): Prepare by adding 500 mL of deionized water into a 1000-mL volumetric flask. Add 0.1 mL each of methanol, ethanol, isopropanol, and acetone. Bring to the mark with deionized water. Store refrigerated in a tightly-sealed glass or plastic container. Stable for at least two years. This Injection Solution may be analyzed as the Positive Control for the assay. Another suitable positive control may be analyzed, as appropriate.

h. Whole Blood Volatiles Control:
Purchased from Cliniqa. Contains acetone, ethanol, isopropanol and methanol. Two levels are typically purchased for ethanol quantitations; either may be used. Concentrations of these analytes differ from lot to lot. Storage conditions and stability determined by manufacturer. This Volatiles Control may be analyzed as the Positive Control for the assay. Another suitable positive control may be analyzed, as appropriate.

i. Negative Control:
An appropriate negative matrix should be used as the Negative Control. In the absence of a more appropriate matrix, deionized water may serve as the negative control for this analysis. A Negative Control will be analyzed with every volatiles assay.

## 7 Calibration

Not applicable.

## 8 Sampling

Not applicable.

## 9 Procedure

Appendix 1 contains an abbreviated version of this procedure. This form may be used at the bench by the examiner or chemist performing the procedure.

a. Into properly-labeled 20-mL headspace vials add 0.5 g sodium chloride or 2.0 mL saturated sodium chloride solution. Add 0.5 mL (or 0.5 g) of specimen or control. Add 0.5 mL Acetonitrile Internal Standard Solution. Alternatively, half these amounts may be added to 10-mL headspace vials. Notes: Unknown liquids may have to be diluted before analysis. The acetonitrile internal standard solution may be omitted or substituted if acetonitrile is a suspected target analyte. All samples in a batch should be prepared the same way.

b. Immediately cap.

c. Vortex sample for 10 seconds.

d. Analyze specimens by headspace GC/MS(EI) after confirming that the instrument is calibrated and in proper working condition.

## 10 Instrumental Conditions

Appendix 2 contains an abbreviated version of HS-GC/MS instrumental parameters used in this procedure that may be used at the bench to verify instrumental parameters.

### 10.1 Headspace Sampler Parameters

| | | | |
|---|---|---|---|
| incubation temperature | 80°C | syringe temperature | 90°C |
| incubation time | 30min | sample fill volume | 1.0mL |
| agitator speed | 300 RPM | sample fill rate | 0.5 mL/sec |
| agitation timing | 10 sec on<br>1 sec off | sample fill strokes | 5 |
| cycle time | 48 min | sample injection speed | 1.0 mL/sec |
| | | syringe flush time | 4.0min |

### 10.2 Gas Chromatograph Parameters

| Oven Parameters | | Column Parameters | | Inlet and Carrier Parameters | |
|---|---|---|---|---|---|
| temperature 1 | 50°C | type | DB-624 | inlet temp. | 150°C |
| hold 1 | 3 min | length | 30 m | injection mode | split |
| ramp 1 | 10°C/min | internal diameter | 0.25 mm | carrier gas | ultrapure helium |
| temperature 2 | 250°C | film thickness | 1.4 µm | carrier mode | constant pressure |
| hold 2 | 21.5 min | | | pressure | 6.54 psi |
| total run time | 44.5 min | | | split ratio | 10:1 |

### 10.3 Mass Spectrometer Parameters

| | | | |
|---|---|---|---|
| ionization mode | electron impact (+) | source temperature | 230°C |
| scan mode | full scan | transfer line temperature | 260°C |
| scan range | 27 - 400 m/z | quadrupole temperature | 150°C |
| multiplier offset | +106 V | solvent delay | 1.6 min |

## 11 Decision Criteria

The following criteria are used as guidelines in determining the acceptability of the data produced in this assay. In general, compound identification should be based on a comparison of the chromatography and mass spectrometry for the analyte peak of interest with data from a contemporaneously analyzed reference standard, calibrator, or Positive Control.

### 11.1 Batch Acceptance Criteria

No analytes of interest should be detected in the Negative Control. For this purpose, analytes of interest are defined as those analytes that will be reported for this batch.

Each of the analytes in the Positive Control should be detected in the headspace GC/MS data. If a targeted run is being performed for a limited set of analytes, only those analytes need to be detected in the Positive Control.

## 11.2 Unknown Sample Acceptance Criteria

### 11.2.1 Chromatography

The peak of interest should show good chromatographic fidelity, with reasonable peak shape, width, and resolution. In order to be determined acceptable, a chromatographic peak in an unknown sample should compare favorably to a chromatographic peak of the same analyte in a known sample analyzed on the same system in the same or subsequent analytical runs. Additionally, the following two criteria should be met.

#### 11.2.1.1 Retention Time

The retention time of the peak should be within ±2% of the retention time (relative or absolute, as appropriate) obtained from injection of a reference standard or Positive Control. The relative retention times of the components should agree with those listed in the enclosed table within ±2%. If not, the shift in relative retention times should be noted and appropriate corrections made when analyzing the data generated from case specimens.

#### 11.2.1.2 Signal-to-Noise

To justify the existence of a peak, its baseline signal to peak-to-peak noise ratio should exceed 3. Further, the baseline signal for the peak of interest should be at least 10 fold greater than that for any observed peak at similar retention time in a Negative Control or blank injected just prior to the sample.

### 11.2.2 Mass Spectrometry

The mass spectrum of the analyte of interest should match that of a reference standard or Positive Control within a reasonable degree of scientific certainty. See the *Guidelines for Comparison of Mass Spectra* standard operating procedure for further guidance.

Table 1: Approximate Relative Retention Times (RRT) to Acetonitrile on DB - 624 column

| Chemical | RRT | Chemical | RRT | Chemical | RRT |
|---|---|---|---|---|---|
| Acetaldehyde | 0.606 | Diethylamine | 1.203 | 1,4-Dioxane | 2.241 |
| Methanol | 0.629 | Hexane | 1.250 | Isoamyl Alcohol* | 2.584 |
| Pentane | 0.783 | 1-Propanol | 1.321 | Toluene | 2.635 |
| Ethanol | 0.800 | Ethyl Acetate | 1.530 | Ethyl Benzene | 3.305 |
| Diethyl Ether | 0.838 | Chloroform | 1.625 | m/p-Xylene | 3.359 |
| Acetone | 0.919 | n-Butyl Chloride | 1.739 | o-Xylene | 3.542 |
| Isopropanol | 0.951 | Isobutyl Alcohol* | 1.794 | 2,2,2-Trichloroethanol | 3.894 |
| Acetonitrile | 1.000 | Benzene | 1.846 | Octanol | 4.688 |
| Methylene Chloride | 1.057 | Isooctane | 1.875 | Cresol | 5.067 |
| t-Butanol | 1.082 | Butyl Alcohol* | 2.062 | | |

*These alcohols were analyzed as nitrite standards.

## 12 Calculations

See the *Guidelines for Toxicological Quantitations* standard operating procedure (Tox 101) for acceptable practices in calculating quantitative results.

## 13 Uncertainty of Measurement

Not applicable.

## 14 Limitations

a. Limits of Detection:

| Chemical(s) | LOD (%v/v) |
|---|---|
| methylene chloride, benzene | 0.0001 |
| toluene | 0.0002 |
| diethyl ether, t-butanol, ethyl acetate, chloroform, n-butyl chloride, octanol, isomayl alcohol, xylenes, ethyl benzene | 0.0010 |
| methanol, acetone, isopropanol, isobutyl alcohol, 2,2,2-TCE, butyl alcohol | 0.0050 |
| acetaldehyde, pentane, ethanol, hexane, isooctane, 1,4-dioxane, propanol, cresol | 0.0100 |
| diethylamine | 0.1000 |

b. Interferences: None known. Care should be taken when interpreting results from grossly decomposed or putrefied samples, as well as samples that have been embalmed. Severe or

extensive putrefaction will result in the generation of a wide variety of low molecular weight volatile compounds in biological specimens, including ethanol, higher weight n-alcohols, aldehydes, sulfides, mercaptans, and alkylamines.

c. Processed Sample Stability: The following compounds are not stable when processed samples sit for one week at room temperature: methylene chloride, hexane, n-butyl chloride, toluene, octanol, xylenes, and ethyl benzene. Therefore, it is suggested that samples be processed the day of analysis, when possible.

## 15 Safety

Take standard precautions for the handling of chemicals and biological materials. Refer to the *FBI Laboratory Safety Manual* for guidance.

## 16 References

Baselt, R.C., *Disposition of Toxic Drugs and Chemicals in Man*, 7th ed., Biomedical Publications: Foster City, California, 2004.

Moffat, A.C., *Isolation and Identification of Drugs*, 2nd ed., Pharmaceutical Press: London, 1986.

Dubowski, K.M., *Manual for Analysis of Ethanol in Biological Liquids*, 1977.

Foerster, E.H., Garriott, J.C."Analysis for Volatile Compounds in Biological Samples"*, J Anal Tox*, 1981, *5*, 241-248.

Garriott, James, *Medicolegal Aspects of Alcohol*, 3rd ed., Lawyers and Judges Publishing: Tucson, AZ, 1996.

*Guidelines for Comparison of Mass Spectra* (Tox 104); FBI Laboratory Chemistry Unit – Toxicology Subunit SOP Manual.

*Ethanol in Biologicals by Automated Headspace GC-FID / GC-MS(EI*) Standard Operating Procedure (Tox 200)*;* FBI Laboratory Chemistry Unit - Toxicology Subunit SOP Manual.

FBI Laboratory Chemistry Unit – Instrument Operation and Support Subunit SOP Manual.

*FBI Laboratory Safety Manual.*

| Rev. # | Issue Date | History |
|---|---|---|
| 1 | 03/05/10 | Removed "Automated" from title. Minimum sample size was updated to 0.25 mL in Section 4. Section 5 was updated to reflect Agilent GC/MS, direct reader to Tox 103, and include saturated sodium chloride solution. Section 6 was updated to reflect new provider of Whole Blood Volatiles Control. Section 7 was updated to direct reader to Tox 200 for quantitation of ethanol, methanol, acetone and isopropanol. Section 9 was updated to use the option of saturated sodium chloride solution, rather than NaCl. Updated MS scan range in Section 10.3. Added Appendix 2, instrument bench sheet. |
| 2 | 05/17/12 | Removed all quantitative references to the procedure which affected Sections 7, 13 and 16. Updated chromatography criteria in Section 11.1. Updated Table 1 with current validation data. Updated Section 14 with current validation data. |
| 3 | 07/09/14 | Added recipe for saturated sodium chloride solution in Section 5.i. Updated Positive Control analytes in 6.h. In Section 10.3, updated solvent delay to catch entire acetaldehyde peak. Added batch acceptance criteria in Section 11.1, and renumbered subsequent sections. Removed internal standard preparation instructions from Appendix 1. Reformatted Appendix 2 to include all pertinent instrumental parameters. |

**Approval**

Redacted - Signatures on File

## Appendix 1: Abbreviated version of the Volatile Procedure for bench use.

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## Appendix 2: Abbreviated version of the instrumental parameters for bench use.

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